

CLAIMS

1. Method for amplification of a target RNA sequence comprising the following steps:

- 5 (a) annealing a first primer to the target RNA sequence, said first primer comprising a hybridizing sequence, which is complementary to and hybridizes to at least a first segment of the target RNA sequence, operatively associated with a promoter sequence;
- 10 (b) extending said first primer in a reaction catalyzed by a DNA polymerase, forming a first RNA/cDNA hybrid nucleic acid molecule;
- (c) selectively removing the target RNA sequence of the first RNA/cDNA hybrid nucleic acid molecule
- 15 forming a first single stranded cDNA sequence;
- (d) annealing a second primer to the obtained first single stranded cDNA sequence, said second primer comprising a hybridizing sequence which is complementary to and hybridizes to a first segment of
- 20 the first single stranded cDNA sequence;
- (e) extending said second primer in a reaction catalyzed by a DNA polymerase to form a first double stranded DNA molecule; and
- (f) employing the first double stranded DNA molecule
- 25 of step (e) in the preparation of a plurality of RNA transcripts that are complementary to the target RNA sequence in a reaction catalyzed by a DNA-dependent RNA polymerase with specificity for the promoter sequence comprised in the first primer;
- 30 wherein the first primer comprises a hybridizing sequence of 7 to 14 nucleotides, a transcription enhancing sequence, and an anchor which is capable of binding to a second segment of the target RNA sequence, and/or wherein the

second primer comprises a hybridizing sequence of 7 to 14 nucleotides, an amplification enhancing sequence and an anchor which is capable of binding to a second segment of the first single stranded cDNA.

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2. Method according to claim 1, further comprising the steps of:

- (g) annealing the second primer to the RNA transcripts produced in step (f);
- 10 (h) extending the second primer in a reaction catalyzed by the DNA polymerase to form a second RNA/cDNA hybrid nucleic acid molecule;
- (i) selectively removing the RNA of the second RNA/cDNA hybrid molecule to obtain a second single
- 15 stranded cDNA molecule;
- (j) annealing the first primer to the obtained second single stranded cDNA sequence;
- (k) extending the 3' end of the second single stranded cDNA molecule in a reaction catalyzed by the
- 20 DNA polymerase using the first primer as a template to form a second partly double stranded DNA molecule comprising a double stranded promotor site;
- (l) employing the second double stranded DNA molecule of step (k) in the preparation of a plurality of RNA
- 25 transcripts complementary to the target RNA sequence in a reaction catalyzed by the DNA-dependent RNA polymerase with specificity for the promotor sequence in the first primer.

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3. Method as claimed in claim 1 or 2, wherein the first primer comprises, going from the 5' end to the 3' end, an anchor, a transcription enhancing sequence, and a hybridizing sequence consisting of 7 to 14 nucleotides which

are complementary a first segment of the target RNA sequence of 7 to 14 contiguous nucleotides.

4. Method as claimed in claim 1 or 2, wherein the
5 second primer comprises of, going from the 5' end to the 3' end, an anchor, an amplification enhancing sequence, and a hybridizing sequence consisting of 7 to 14 nucleotides which are complementary a the first segment of the first single stranded cDNA sequence of 7 to 14 contiguous nucleotides.

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5. Method as claimed in any of the claims 1-4 wherein the hybridizing sequence comprises 7-10 nucleotides which are complementary a first segment of the target RNA sequence of 7 to 10 contiguous nucleotides..

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6. Method as claimed in any of the claims 1-5, wherein the anchor is an, optionally modified, oligonucleotide, comprising 7 to 22, optionally modified, nucleotides, which binds to the second segment of the target
20 RNA sequence or to the second segment of the first single stranded cDNA molecule.

7. Method as claimed in claim 6, wherein the anchor is an, optionally modified, oligonucleotide, comprising 7 to
25 14, preferably 9 to 14, optionally modified nucleotides.

8. Method as claimed in claim 6 or 7, wherein the anchor comprises DNA, RNA, 2'O-methyl modified nucleotides and/or LNA.

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9. Method as claimed in any of the claims 1-5 wherein the anchor comprises PNA.

10. Method as claimed in any of the claims 1-5,
wherein the anchor comprises a protein, or fragments derived
thereof, which bind(s) to the second segment of the target
RNA sequence or the second segment of the first single
5 stranded cDNA molecule.

11. Method as claimed in claim 10, wherein the
protein, or fragments derived thereof, are chosen from the
group consisting of a RNA binding protein, a polyC-binding
10 protein, a polyA-binding protein and a protein comprising a
zinc-finger, a restriction enzyme, and an antibody, or
fragments thereof.

12. Method as claimed in any of the claims 1-11,
15 wherein the second segment is separated from the first
segment by 0 to 6 nucleotides, preferably by 0 to 4
nucleotides, more preferably by 0 to 3 nucleotides.

13. Method as claimed in any of the claims 1-12,
20 wherein the transcription enhancing sequence reads:
5'-AAACGGGCACGAGC-3'.

14. Method as claimed in any of the claims 1-13,
wherein the amplification enhancing sequence reads:
25 5'-GACTTCAGGACTTCAGG-3'.

15. Method as claimed in any of the preceding claims
1 to 14, wherein the promoter sequence is the bacteriophage
T7 promoter sequence.

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16. Method as claimed in any of the preceding claims
1 to 15, wherein the DNA polymerase is the avian
myeloblastosis virus (AMV) reverse transcriptase.

17. Method as claimed in any of the claims 1-16, wherein the target RNA sequence is a segment of the human immunodeficiency virus (HIV).

5 18. Method as claimed in any of the claims 1-17, wherein the target nucleic acid is a segment of the human hepatitis C virus.

19. Method as claimed in any of the preceding claims
10 1-18, wherein the RNA transcripts are detected by one or more sequence-specific probes.

20. Method as claimed in claim 19, wherein the
sequence-specific probe hybridizes to a sequence identical to
15 the amplification sequence of the second primer.

21. Primer comprising a hybridizing sequence, which
is complementary to and hybridizes to a first segment of a
target RNA sequence, and an anchor binding to a second
20 segment of the target RNA sequence.

22. Primer, comprising, going from the 5' end to the
3' end, an anchor, a transcription enhancing sequence or an
amplification enhancing sequence, and a hybridizing sequence
25 of 7-14 nucleotides, preferably 7-10 nucleotides.

23. Primer as claimed in claim 21 or 22, wherein the
anchor is an, optionally modified, oligonucleotide,
comprising 7 to 22, optionally modified, nucleotides, which
30 bind to the second segment of the target RNA sequence or to
the second segment of the first single stranded cDNA
molecule.

24. Primer as claimed in claim 23, wherein the anchor is an, optionally modified, oligonucleotide, comprising 7 to 14, preferably 9 to 14, optionally modified nucleotides.

5 25. Primer as claimed in claim 21 or 22, wherein the anchor comprises DNA, RNA, 2'-O-methyl modified nucleotides and/or LNA nucleotides.

 26. Primer as claimed in claim 21 or 22, wherein the
10 anchor comprises PNA.

 27. Primer as claimed in claim 21 or 22, wherein the anchor comprises a protein, or fragments derived thereof, which are capable of specific binding to the second segment
15 of the target RNA sequence or the second segment of the first single stranded cDNA sequence.

 28. Primer as claimed in claim 28, wherein the protein, or fragments derived thereof, are chosen from the
20 group consisting of an RNA binding protein, a polyC-binding protein, a polyA-binding protein and a protein comprising a zinc-finger, a restriction enzyme, and an antibody or fragments thereof.

25 29. Primer as claimed in any of the claims 21-29, wherein the transcription enhancing sequence reads
5'-AAACGGGCACGAGC-3'.

 30. Primer as claimed in any of the claims 21-30,
30 wherein the amplification enhancing sequence reads
5'-GACTTCAGGACTTCAGG-3'.

31. Primer as claimed in any of the claims 21-31, wherein the promotor sequence is the bacteriophage T7 promotor sequence.

5 32. Kit for the amplification and/or detection of a target RNA sequence, comprising at least one or more primers as claimed in claims 21-32.

10 33. Kit as claimed in claim 33, further comprising one or more sequence-specific probes, an amplification buffer, and/or one or more enzymes.